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Hormonal Control of the Sexually Dimorphic Pigmentation of *Thalassoma bifasciatum*[1]

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(Plates I-III)

THE bluehead, *Thalassoma bifasciatum* (Bloch), shows the blue-headed condition only in the adult males while immature fish and females show quite other colors and patterns. The fact that the males are sexually mature some time before they develop their strikingly different secondary sex characters has led to a considerable taxonomic confusion. The females and young have gone under the name of *T. nitida* (Günther) and *T. nitidissima* (Goode), depending on the color phase they showed. The males have been referred to as *T. subfurcatus* Nichols. This situation was recognized by Longley (1914, 1915) but not generally accepted by ichthyologists. As recently as 1930 Nichols still considered the possibility of them being separate species. Breder (1927) and Beebe & Tee-Van (1928) were disposed to the view that they were all one species. Tee-Van (1932) referred *T. nitidum* and *T. subfurcatus* Nichols to the synonomy of *T. bifasciatum*. *T. nitidissima* was added to the synonomy by Beebe & Tee-Van (1933).

The coloration and pattern of the juvenile, female and young male *Thalassoma* is principally that of a yellow-backed fish with or without a dark lateral stripe. This stripe may be broken up into blocks. Changes in color patterns are observable in the living individuals. Variations of this type of pattern are not the concern of the present study, however.

The presence of males with two different colorations in a single species suggested that the factor instituting the change from small yellow male to larger blue male was hormonal rather than chromosomal. If this were the mechanism, injections of sufficient androgenic hormone given to yellow-phase fish should cause the blue phase to replace it and would clearly establish these two forms as individuals of the same species, accounting for the presence of two types of males and only one type of female.

Darby (1935-1936) reported Testosteron (Ciba) effective in causing " . . . the immature bluehead (*Thalassophryne sp.*)" [*sic*] to take on mature coloration. The designation of the fish used as *Thalassophryne* sp. is probably incorrect as this form is a genus of toadfishes found in the Pacific. There is one report (Günther, 1861) of a single Atlantic species, *Thalassophryne maculosa* Günther, from Panama, but this report is in question. There have been no reports of *Thalassophryne* sp. in the vicinity of the Dry Tortugas. *Thalassoma* was probably the "bluehead" used. No subsequent publication on the problem has been found.

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MATERIALS AND METHODS

The blueheads used in this investigation were collected from tidal flat *Thallasia* beds and around small coral growths in Bimini, Bahamas, B. W. I. Experimental work was done in the Lerner Marine Laboratory at Bimini. While under observation, the fish were kept in aquaria with running sea water. Conch shells were provided for shelter.

Four groups of fish were used. Group 1 comprised "nitidum" and "bifasciatum" fish collected in February and March. "Nitidum" and "bifasciatum" designate color phases and are described in a separate section. These fish were used for study of normal tissue. Group 2 consisted of "nitidum" fish used in a preliminary experiment to determine dosage and medium for suspension of the hormone. Methyl testosterone

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was dissolved in 95% alcohol and suspended in physiological saline. Injections were given intraperitoneally without anesthesia. Dosage was not equal for all fish, as the saline caused a partial precipitation of the hormone and additional 95% alcohol was added to restore the suspension. The alcohol was found to be toxic and fatalities were numerous. Another set of fish was designated as Group 3. In this case methyl testosterone was dissolved in 95% alcohol, suspended in sesame oil and incubated at 37° C. to evaporate the alcohol. A 1% solution of ethyl urethane dissolved in sea water was used as an anesthetic. A group of fish was collected in June for comparison with Group 1. These fish were designated as Group 4. The ratio of males to females in June differed from the ratio in the February-March period. In March, 28 fish were collected of which nine were males and 19 females. Two to three months later, 18 fish were collected and ten were males, seven females and one a true hermaphrodite having one lobe of testicular tissue and one of ovarian tissue (Pl. III, Figs. 15 & 16).

The numbers of fish, dosages and descriptions of fish in all groups are given in Table 1 with dates of collection, treatment and sacrifice.

All fish were killed by a lethal dose of ethyl urethane. This caused the dispersion of melanin granules, making the fish assume their darkest coloration. Photographs in Pl. I were taken after the fish had been killed, in order to show them uniformly in this darkest and most marked pattern.

Bouin's solution was used for fixation. The gonads were dissected from all fish, imbedded in paraffin and sectioned at 7μ. Sections were stained with Harris's hematoxylin and eosin and with Masson's trichrome stain for connective tissue.

Observations

Color and Pattern.—The predominant color of the "nitidum" stage is yellow. A dark median lateral stripe marks the fish from the snout to the caudal fin, running through the eye. The stripe is sometimes modified into six rectangular areas separated by gray or yellow bars. These intermediate markings are the basis for the distinction between "nitidum" and "nitidissimum," the former notably displaying the gray and the latter the yellow. In "nitidum," the dorsal fin is bordered by light blue and the caudal fin has a dark margin. In shape, the caudal fin is slightly concave as the median rays are shorter than the peripheral rays. Ventral and anal fins are transparent but the pectoral fins have slightly dusky tips. A fish in the "nitidum" stage is shown in Pl. I, Fig. 1.

"Nitidissimum" would appear to be a modification of "nitidum" in which bands of yellow replace the gray ones. This stage is more apt to be a product of activity and environment than of age or sex. Longley & Hildebrand noted that this phase was shown most frequently by resting fish. For the purposes of this study, "nitidissimum" fish are included in the "nitidum" classification.

Blue is the principal color of the "bifasciatum" stage. Longley & Hildebrand described a blue or violet coloration on the head and the throat extending to the base of the dorsal fin. Fish in the "bifasciatum" phase kept in the aquaria seldom showed the violet coloration but the blue was vivid. The body of the fish is banded by two black bars situated behind the pectoral fins. The black bars are separated by a blue bar. The remainder of the body of the fish from the second black bar is greenish. As in the case of the violet color, green is seldom observed to be displayed by fish in aquaria; blue or yellow is the color shown. The pectoral fins have distinct black tips. The caudal fin is markedly forked. The black bars of the body extend through the dorsal fin which is otherwise transparent. Fish in this phase are larger than "nitidum" phase fish. Pl. I, Fig. 4, pictures an untreated "bifasciatum" fish.

The injection of methyl testosterone produced "bifasciatum" coloration and pattern in "nitidum" phase fish regardless of sex. Pl. I, Fig. 2, shows a treated male; Fig. 3 shows a treated female. The first sign of the "bifasciatum" coloration became apparent four days after injection. During these four days the yellow color and "nitidum" pattern gradually faded. The blue coloration started in the head region and three days later the black bars in the region of the pectoral fins began to appear. The initial black of the bars originated at the point where the bar would have bisected the stripe of the "nitidum" phase. The tips of the pectoral fins which cover this region became darker because of an increase in the number of melanophores. Pl. II, Figs. 5 and 6 show the difference in the amount of pectoral fin pigmentation between a "nitidum" stage and an experimentally-induced "bifasciatum" stage. In the ten days following the appearance of the black bar, the blue coloration spread over the entire body of the fish, gradually increasing in intensity.

In the aquaria, the fish were light blue and the "bifasciatum" pattern was evident but not intense. When sacrificed in urethane with the resulting full expansion of the pigment cells, the fish were vivid blue and the pattern well defined in all but two of the 15 fish. These two fish were blue but showed both faint black bars and faint black stripes. The experimentally-induced blue color, however, was never as intense as the blue

TABLE 1. SUMMARY OF FOUR GROUPS OF *Thalassoma bifasciatum*.

	Date collected	Phase	Number	Average standard length in mm. at time of sacrifice	Average total length in mm. at time of sacrifice	Treatment Date–Type	Date of sacrifice	Gonads
Group 1	March 4	bifasciatum	12	84	103	None	March 4	Testes 12
	Feb. 19	nitidum	9	65	75	None	Feb. 19	Testes 4 Ovaries 5
Group 2	Feb. 18	nitidum	16			Feb. 18, 1 mg. methyl testosterone		
			15			Feb. 25, 1 mg. methyl testosterone		
			3	64	74	March 8, 2 mg. methyl testosterone	March 26	Ovotestes 3
	Feb. 25	nitidum	6	59	69	Feb. 25, 0.1 cc. 95% alcohol	March 26	Testes 2 Ovaries 1
Group 3	March 6	nitidum	16	63	72	March 6, 0.5 cc. sesame oil	March 26	Testes 2 Ovaries 14
		nitidum	15	61	71	March 6, 2 mg. methyl testosterone	March 26	Testes 2 Ovotestes 13
Group 4	Early June	bifasciatum	6	78	94	None	Same day as collected	Testes 6
		nitidum	18	60	70	None		Testes 10 Ovaries 7 Hermaph-rodite 1

displayed by the wild fish in the "bifasciatum" phase. Comparison of the wild and the experimental "bifasciatum" coloration showed that the green element was lacking in the experimentals.

Goodrich & Biesinger (1953), working on the histology of the coloration of *Thallasoma bifasciatum,* noted that the underlying tissue of the green scales had a layer of guanophores as well as the xanthophores and melanophores present in the black and the blue areas. The number of melanophores present in the green areas was less than the number present for the other two colors: 200 per sq. mm. in green areas and 450-500 per sq. mm. in black areas. However, the number of xanthophores present in the green areas was greater than that present in black or blue areas: 350 per sq. mm. for green, 15 scattered per sq. mm. for blue and 45 per sq. mm. for black. All the numbers given were approximations.

There are two possible explanations for the nonappearance of the green color: (1), that the two weeks' time the experimental fish were maintained after injection was not long enough to build up the concentration of xanthophores and guanophores necessary to produce green coloration; and (2), that an improper diet caused loss of color in the xanthophores (Fox, 1953). The one fish from Group 2 which was maintained for more than six weeks did show some green coloration when anesthetized in ethyl urethane.

Histology.—In histological section, distinction could be made between normal "nitidum" testes and normal "bifasciatum" testes. The testes of males in the "nitidum" phase contained large reservoirs of mature sperm aggregated in the lumen of the sperm duct and held in the tubules. The bulk of the testes was comprised of large cysts with thin membranes containing mature sperm. Very few early or intermediate stages of spermatogenesis could be noted (Pl. II, Fig. 7).

Small reservoirs of mature sperm were seen in the tubules of "bifasciatum" testes. The greater part of the testes, however, was made up of cysts in early or intermediate stages of spermatogenesis. A few cysts were present which contained mature sperm and these appeared to be in the stage immediately preceding rupture. The sperm cells were clustered on or near the walls of the cysts while the centers of the cysts were empty (Pl. II, Fig. 8). The testes of fish in this stage were half as large as testes from fish in the "nitidum" phase. No interstitial cells like those described by Courrier (1921) were identified. The specific source of the androgenic hormone responsible for the blue color and pattern is as yet unknown.

The presence of large quantities of sperm in the testes of "nitidum" males indicates that sexual maturity for the males is attained in this phase. The absence of large sperm reservoirs in "bifasciatum" fish suggests that some spawning has occurred during the "nitidum" phase to deplete the sperm reservoirs.

Normal ovaries showed early and intermediate maturation stages as well as mature eggs. The follicles were large and the ova were surrounded by heavy chorionic membranes and encircled by follicular cells (Pl. II, Fig. 9).

Sesame oil injections used for control purposes produced variations from the normal in both "nitidum" males and females. The testes of the two males of the group showed all mature sperm, indicating that spermatogenesis had been accelerated. The sperm were held within the cysts and the membranes were well-defined and thick. Only a few small sperm reservoirs could be seen. Spaces formerly occupied by the sesame oil which had been dissolved by the histological procedure were scattered throughout the gonads (Pl. II, Fig. 10). The size of the testis was not affected by the sesame oil.

Ovaries from sesame oil-injected females were about one-quarter the size of the normal ovaries. Each ovary was sac-like in structure and enclosed a large empty lumen. The walls of the sac were as thin as three to four cell layers in some fish. No mature eggs were seen; however, loose follicular cells were present. Very little connective tissue was seen. A few chorionic membranes were observed, which suggested that some eggs had been resorbed (Pl. III, Figs. 11 & 12).

The testes of males injected with methyl testosterone showed only mature sperm. The membranes of the cysts were thin and there were large reservoirs of mature sperm (Pl. III, Fig. 13). The androgenic hormone accelerated the production of mature sperm. Early and intermediate stages of maturation were absent.

The gonads of the 13 other fish which had received injections of methyl testosterone showed ovarian tissue interspersed with maturation stages of spermatogenesis (Pl. III, Fig. 14). The predominance of ovarian tissue indicates that these fish were females before injection. The ovaries were drastically reduced in size to less than a quarter the size of ovaries of normal females collected at the same time and location. The ovary appeared as a sac-like structure, hollow and collapsed. In section, maturation stages, mature eggs and degenerating eggs could be seen. Collapsed chorionic membranes were plentiful and there was an abundance of connective tissue. All stages of spermatogenesis could be identified in the ovaries and small res-

ervoirs of mature sperm were present. The testicular tissue was present throughout the organ and did not appear to be especially abundant in any specific part. This tissue is thought to have arisen from primordial germ cells.

Discussion

Previous experiments with sex reversal in fishes have been carried out primarily on representatives of the poeciliid fishes. Particular emphasis has been given to *Xiphophorus helleri* (Heckel). The development in the female of the secondary sex characters of the male of this species has served as an external index of possible sex reversal.

Essenberg (1926) reported spontaneous sex reversal from female to male in *X. helleri.* The reversal occurred even after the female had given birth to one or more broods. After reversal the secondary sex characters of the male were exhibited by the fish but the female shape was retained. On the basis of these observations, Essenberg concluded that sex in this species was determined and controlled hormonally and not genetically and stated that any agent or condition which tends to decrease the capacity of the female sex hormone secretion beyond a certain limit becomes an immediate factor in sex reversal in the female.

In 1937, Witschi & Crown found that nonpregnant female *X. helleri* subjected to testosterone propionate (Ciba) dissolved in the aquarium water, absorbed their eggs and the ovaries resembled testes although no spermatogenesis was observed. The secondary sex characters of the male were displayed. Pregnant females under similar treatment aborted or absorbed their eggs within one to two days.

Baldwin & Goldin (1939) reported histological changes in ovaries of 50% of a group of virgin female *X. helleri* injected with testosterone propionate dissolved in sesame oil. The changes included absorption of the gonad and the presence of some phases of spermatogenesis. Baldwin & Li (1942) demonstrated the possibility of complete sex reversal in adult female *X. helleri* treated with gonadotrophic (human chorionic) hormone, and later (1945) cited two cases of ovotestes in males that had been injected with alpha-estradiol benzoate.

Burger (1942) treated male *Fundulus* with testosterone propionate and found that it had only a slight stimulating effect on the male germ cells. An increased coloration was noted and an increase in the extent of the testicular duct system.

Mature female *Gambusia holbrooki* Girard developed masculinized anal fans when treated with testosterone propionate. Immature females showed inhibition of growth (Hamon, 1946). Female *Gambusia affinis* maintained in a solution of ethynyl testosterone for two to four days developed masculine secondary sex characters and retained these characters up to 60 days after they had been replaced in fresh water. On the basis of this experiment, Turner (1946) stated that this response was a clear indication that the genetic factors for the male characters were present in the female but normally did not develop because of insufficient androgenic hormone.

Zei (1950) described typical sex reversal in three Mediterranean fishes, *Maena smaris* (Linnaeus), *M. chryselis* (Cuvier & Valenciennes) and *Pagellus erythrinus* (Cuvier & Valenciennes). The fish are female for the first half of their lives and male the second. Transformation takes place at the 13-15 cm. size. The males may be identified by brighter color, larger size and better developed anal and dorsal fins.

The histology of hermaphroditism in *Serranus* and *Sargus* was described by Van Oordt (1929). Lavenda (1949) reported the presence of developing testicular tissue in functional female sea bass, *Centropristes striatus.* The testicular tissue arose from the epithelial lining of the oviduct.

According to D'Ancona (1950) hermaphroditism in teleosts is found only in some species of *Sparida* and *Serranida.* In the case of the Sparidae, he assumes two different germinal areas for gonad origination reaching maturity successively and each producing distinct sex differentiators known as *gynogenine* and *androgenine.*

Courrier (1921) described interstitial cells in the testes of representatives of the Gobiidae, Callionymidae, Cottidae, Cichlidae and Gasterosteidae. The function of control of the secondary sex characters was ascribed to the interstitial cells. Van Oordt (1925) found no interstitial cells in the testis of *X. helleri* (Heckel). Craig-Bennett (1931), studying *Gasterosteus aculeatus* Linnaeus, found interstitial cells most abundant during the breeding season and inconspicuous in quiescence.

The existence of teleost androgens has been experimentally demonstrated. In 1937, Hazleton & Goodrich accelerated comb growth of two capons with an extract of the testes of salmon, *Oncorhynchus kisutch.* Potter & Hoar (1954) reported androgens in the testes of *Oncorhynchus keta* Walbaum. Histological examination of the testes showed that interstitial cells and extracts produced comb growth in baby chicks. The number of interstitial cells was dependent on the season, as with *Gasterosteus aculeatus* Linnaeus.

Results of the present report show that *T. bifasciatum* is not a hermaphroditic fish but is possibly a progynous species in which all individuals start life as female and later become male. Development of the "bifasciatum" phase is under the control of the androgenic hormone as injection of male hormone brought about the male coloration regardless of the sex of the "nitidum" fish used. However, the appearance of the blue, "bifasciatum" coloration is not concomitant with sexual maturity, because mature testes are found in the yellow phase, but it may be a function of age and length of time the androgenic hormone has been in action.

The sac-like structure and reduced size of the ovary of the hormone-treated fish may be the result of the sesame oil in which the hormone was suspended. The ovaries of females treated with sesame oil alone showed a similar modification in size and structure. It is of interest to note that the sesame oil was inert in regard to testicular tissue and caused no change in size or structure of testes.

Summary

1. All *Thalassoma bifasciatum* with blue heads are males but fish with largely yellow coloration are male, female or juvenile.

2. Large reservoirs of mature sperm were found in testes of yellow males, indicating that sexual maturity is reached in this coloration phase. The testes of blue males contained numerous early and intermediate stages of spermatogenesis but very few mature sperm. The testes of yellow males were twice as large as the testes of blue males.

3. Intraperitoneal injection of methyl testosterone in yellow-phase fish produced blue-phase color and pattern in both sexes.

4. Androgenic hormone is responsible for the change from yellow to blue phase. The first indication of color change was noted on the head region four days after injection, the first indication of pattern change was observed three days later, on and under the tips of the pectoral fins with an increase in the number of melanophores present.

5. Methyl testosterone produced the development of testicular tissue and the regression and absorption of ovarian tissue in yellow females and acceleration in the production of sperm in yellow males.

6. Testicular tissue that developed in yellow females evidently arose from the primordial germ cells.

7. The experimentally produced ovotestes contained large amounts of connective tissue absent in the gonads of both normal females and males.

8. No interstitial cells for androgenic hormone elaboration could be found in testes from blue-phase fish.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Female bluehead in the "nitidum" phase. × .71.

Fig. 2. "Nitidum"-phase male three weeks after injection of 2 mg. methyl testosterone. The black lateral stripe has been obliterated and the color and pattern of the "bifasciatum" stage has begun to appear. × .74.

Fig. 3. Female "nitidum"-phase fish after injection of 4 mg. methyl testosterone, showing pronounced "bifasciatum" color and pattern. The blue color is indistinguishable in black and white reproduction. × .71.

Fig. 4. Untreated male in "bifasciatum" phase. × .84.

Plate II

Fig. 5. Tip of pectoral fin of a "nitidum"-phase fish. × 100.

Fig. 6. Tip of pectoral fin from a fish in an experimentally-produced "bifasciatum" phase. × 100.

Fig. 7. Testis of untreated "nitidum"-phase fish showing relatively large numbers of mature sperm and a few maturation stages. × 600.

Fig. 8. Testis of untreated "bifasciatum"-phase fish showing predominance of early stages of spermatogenesis and relatively few mature sperm. × 600.

Fig. 9. Ovary of untreated "nitidum" fish. × 90.

Fig. 10. Testis of "nitidum"-phase fish three weeks after injection of 0.5 cc. sesame oil. × 100.

Plate III

Fig. 11. Ovary three weeks after injection of 0.5 cc. sesame oil, showing decrease in size of the organ and decrease in size and number of eggs. × 100.

Fig. 12. Detail of Figure 11, showing collapsed chorionic membrane. × 600.

Fig. 13. Testis after injection of 2 mg. methyl testosterone, showing only mature sperm and no stages of early spermatogenesis. × 600.

Fig. 14. Gonad, designated as an ovotestis, three weeks after injection of 2 mg. methyl testosterone. Note early stages of spermatogenesis and degenerating ova. × 600.

Fig. 15. One lobe of gonad of hermaphroditic "nitidum" fish, showing only testicular tissue. × 110.

Fig. 16. The other lobe of the gonad pictured in Figure 15, showing typical ovarian structure. × 110.